REMARKS

Reconsideration of the above-identified application in view of the amendment above and the remarks below is respectfully requested.

Claims 2-5, 8, 16 and 21-23 have been canceled in this paper. Claims 1, 6-7, 9-15 and 17-20 have been amended in this paper. No new claims have been added in this paper. Therefore, claims 1, 6-7, 9-15 and 17-20 are pending and are under active consideration.

In the outstanding Office Action, the Patent Office repeats the following restriction requirement, which was communicated previously to the undersigned by telephone on or about October 31, 2008:

> Group I, claim(s) 1-20, drawn to a method for the analysis of cytosine methylation.
>
> Group II, claim(s) 21-23, drawn to a kit comprising a probe, a repair enzyme, a polymerase, and additional reagents necessary for PCR.

Applicants hereby affirm their election of Group I, claims 1-20.

Also in the outstanding Office Action, the Patent Office states that the Information Disclosure Statement submitted on July 10, 2006 "is in compliance with the provisions of 37 CFR 1.97" and that "the information disclosure statement is being considered by the examiner." Notwithstanding the above, the Patent Office also states that "[t]he documents under 'Foreign Patent Documents,' are not being considered as they are in a foreign language."

In response, Applicants respectfully submit that the Patent Office's decision not to consider the foreign patent documents, namely, DE19853398C1 and DE10204566A1, was in error as the

documents in question should have been considered. According to 37 CFR 1.98, foreign-language documents that are included in an Information Disclosure Statement are to be considered by the Patent Office if the Information Disclosure Statement includes a concise explanation of the relevance of the documents. As explained in MPEP 609.04(a), III, the requirement for a concise explanation of the relevance of a document may be satisfied (i) by providing a translation of the document or by providing an English-language abstract of the document **or** (ii) by submitting an English-language version of a search report from a foreign patent office which indicates the degree of relevance found by the foreign patent office. In the instant case, Applicants submitted with the Information Disclosure Statement an English-language copy of the International Search Report from the corresponding PCT application and referenced this fact in the subject Information Disclosure Statement. The International Search Report submitted by Applicants indicates the degree of relevance of the documents in question. In view of the above, it can clearly be seen that Applicants have complied with the outstanding requirements for consideration of foreign-language documents. Therefore, Applicants respectfully request that the Patent Office consider the documents in question.

Claim 12 stands objected to because "'a DNA repair enzyme are utilized,' is not proper grammar."

Without acquiescing in the propriety of the objection, Applicants note that claim 12 has been amended to obviate the objection. Therefore, the objection should be withdrawn.

Claims 17 and 18 stand objected to under 37 CFR 1.75(c) "as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite

the claim(s) in independent form." In support of the objection, the Patent Office states that "[t]he claims recite an intended use of the method of claim 1, without reciting any positive active steps which would further limit the method."

Without acquiescing in the propriety of the objection, Applicants have rewritten claims 17 and 18 in independent form. Therefore, the objection has been rendered moot and should be withdrawn.

Claims 1-20 stand rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> Claims 1-20 are confusing because it cannot be determined what is encompassed by the term, "characterized in that." The scope of the phrase is unclear, and it is suggested to use conventional U.S. claim language, such as "comprising," or "wherein."
>
> Claims 1-20 are confusing because claims 1, 4, 5, 7, 9-10, 12, and 14-16 do not recite any active steps. For instance, "is hybridized," is not considered a positive, active step. While minute details are not required in method claims, at least the basic steps must be recited in a positive, active fashion. See Ex parte Erlich, 3 USPQ2d, p. 1011 (Bd. Pat. App. Int. 1986).
>
> Claim 5-7 are confusing because it cannot be determined what is encompassed by "background DNA," first cited in claim 5, and also appears in numerous dependent claims. It is unclear from the claim and specification what is encompassed by this type of DNA. Furthermore, not providing such description also renders the invention unclear, so it is suggested to provide further clarification.
>
> Claim 5 recites the limitation "the background DNA." There is insufficient antecedent basis for this limitation in the claim.
>
> Claim 7 is confusing because it cannot be determined what is encompassed by "the oligonucleotides utilized in step b) are simultaneously utilized as primers or probes in a later amplification

> step." The phrase is unclear as written, since first, it is confusing how the oligonucleotides are simultaneously utilized, and second it is unclear how they can be *simultaneously* utilized in a *later* amplification step. Clarification is required. (Emphasis in original.)

Applicants respectfully traverse the subject rejection. Insofar as the rejection is predicated on the use of the expression "characterized in that," this ground for the rejection has been overcome as this expression is no longer recited in the claims. Insofar as the rejection is predicated on an alleged lack of positively-recited active steps in the claims, Applicants respectfully submit that the claims are now largely written in the active voice. Insofar as the rejection is predicated on an alleged lack of definition or antecedent basis for the term "background DNA," Applicants respectfully submit that these grounds have been overcome. Insofar as the rejection is predicated on the use of the term "simultaneously" in claim 7, this ground for the rejection has been overcome as this term is no longer recited in claim 7.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-6, 12 and 17-18 stand rejected under 35 U.S.C. 102(b) "as being anticipated by Berlin (AU 200018565 B2, published June 2000)." In support of the rejection, the Patent Office states the following:

> Regarding claim 1, Berlin teaches a method for the analysis of cytosine methylation, characterized in that:
>
> a) the DNA to be investigated is chemically or enzymatically converted so that 5-methylcytosine remains unchanged, while unmethylated cytosine is converted to uracil or to another base which differs from cytosine in its base pairing behavior (see pg. 6, pg. 7, second full paragraph; bottom half; pg. 11, last paragraph),
>
> b) the converted DNA is hybridized with oligonucleotides, whereby the DNA of one methylation status forms hybrids with erroneous base pairings, while the DNA of the other methylation status forms hybrids without erroneous base pairings or does not form

hybrids (see pg .6, bottom half; pg. 8, first half; pg. 12, last paragraph),

c) one strand of the erroneously paired hybrids is enzymatically cleaved (see pg. 6 – step c); pg. 10, first half; pg.13, second to last paragraph).

d) the uncleaved DNA or the cleaved fragments are detected (pg. 7 – step e); pg. 8, bottom half; pg.10, bottom half; pg. 14, top half),

e) the methylation status of the investigated DNA is concluded from the detection signal generated in step d)(see pg. 7 – step e); pg. 8, bottom half; pg. 10, bottom half; pg. 14, top half and examples).

Regarding claims 2-3, Berlin teaches the method further characterized in that in step b), the DNA of one methylation status forms hybrids with erroneous base pairings, while the DNA of the other methylation status forms hybrids without erroneous base pairings, or does not form hybrids (see pg. 8, first half; pg. 12, second half – which discusses erroneous base pairings in the heteroduplexes formed, the reaction also inherently includes situations where some hybrids do not form).

Regarding claim 4, Berlin teaches a method for the analysis of cytosine methylation, characterized in that:

a) the DNA to be investigated is chemically or enzymatically converted so that 5-methylcytosine remains unchanged, while unmethylated cytosine is converted to uracil or to another base which differs from cytosine in its base pairing behavior,

b) the converted DNA is hybridized to oligonucleotides, whereby the DNA to be detected forms hybrids with erroneous base pairings,

c) the oligonucleotide strand of the erroneously paired hybrids is enzymatically cleaved,

d) the cleaved oligonucleotide fragments are detected,

e) the methylation status of the investigated DNA is concluded from the detection signal generated in step d)(see claim 1 for Berlin's teachings).

Regarding claim 5, Berlin teaches a method for the analysis of cytosine methylation, characterized in that:

a) the DNA to be investigated is chemically or enzymatically converted so that 5-methylcytosine remains unchanged, while

unmethylated cytosine is converted to uracil or to another base which differs from cytosine in its base pairing behavior,

b) the converted DNA is hybridized to oligonucleotides, whereby the background DNA forms hybrids with erroneous base pairings,

c) the DNA strand of the erroneously paired hybrids is enzymatically cleaved,

d) the uncleaved DNA is detected,

e) the methylation status of the investigated DNA is concluded from the detection signal generated in step d)(See claim 1 for Berlin's teachings, and also pg. 8, where hybrids form duplexes in unchanged DNA, which is subjected to cleavage in the invention (and will not be cleaved), and then detected by mass spectrometry by size.

Regarding claim 6, Berlin teaches the method further characterized in that the background DNA forms several erroneous base pairings with the oligonucleotides (see pg. 12, second half - which discusses erroneous base pairings in the heteroduplexes formed).

Regarding claim 12, Berlin teaches the method further characterized in that in step c) a DNA repair enzyme are utilized (see pg. 6 – step c); pg. 10, first half; pg. 13, second to last paragraph, Muts).

It is noted that due to the indefiniteness of claims 17-18, it cannot be determined how the instant invention differs from the prior art.

Insofar as the subject rejection relates to claims 2-5, the rejection is moot in view of Applicants' cancellation of these claims in this paper. Insofar as the subject rejection relates to claims 1, 6, 12 and 17-18, Applicants respectfully traverse the subject rejection.

Claim 1, from which claims 6 and 12 depend, has been amended in this paper and now recites "[a] method for the analysis of methylated DNA as compared to background DNA of the same sequence but another methylation pattern comprising:

a) converting the DNA to be investigated chemically or enzymatically so that 5-methylcytosine remains unchanged, while unmethylated cytosine is converted to uracil or to another base which differs from cytosine in its base pairing behavior,

b) hybridizing the converted DNA with oligonucleotides, whereby the background DNA forms hybrids with erroneous base pairings,

c) cleaving the DNA strand of the erroneously paired hybrids enzymatically,

d) amplifying the uncleaved DNA,

e) detecting the amplificates,

f) concluding the methylation status of the investigated DNA from the detection signal generated in step e).

g) wherein steps b) through d) are conducted simultaneously."

The term "background DNA," which is recited in the preamble of claim 1, is disclosed in the present specification, for example, on page 2, first paragraph. The newly introduced features of "amplifying the uncleaved DNA" (in step (d)) and "detecting the amplificate" (in step (e)) are disclosed in the present specification, for example, on page 21, second paragraph. The newly introduced feature that steps (b) through (d) are conducted simultaneously is disclosed in the present specification, for example, on page 23, last paragraph, as well as in original claim 16.

As amended, claim 1 is now directed at a method in which the so-called background DNA, i.e., DNA of the same sequence as the methylated DNA that is to be detected but with another methylation pattern, is degraded during amplification of the methylated DNA that is to be analyzed. Claim 1 is neither anticipated by nor rendered obvious over Berlin for at least the reason that Berlin

neither teaches nor suggests, amongst other things, the performance of steps b) through d) simultaneously, i.e., hybridizing all oligonucleotides to the converted DNA such that the background DNA forms hybrids with erroneous base pairings, cleaving the DNA strand of the erroneously paired hybrids enzymatically, and, at the same time, amplifying the uncleaved DNA.

With regard to the issue of nonobviousness, it should be noted that the performance of steps b) through d) in a simultaneous manner reduces the amount of so-called "background DNA" and, therefore, increases the sensitivity and accuracy of the analysis of methylated DNA. This feature is not taught or suggested by Berlin.

Instead, Berlin describes a method in which hybrids of DNA strands are formed that stem from two different sources. Berlin discloses amplifying the DNA of these different sources individually and then jointly treating them by introducing a detectable label (see Berlin at page 7, paragraph 5, together with paragraph 1). This teaches away from the present invention, in which an amplification reaction is not only used to amplify DNA, but also to eliminate background DNA. The problem of background DNA and possible ways to reduce it, however, are not mentioned in Berlin. Therefore, a person of ordinary skill in the art would not have been able to obtain any hints from Berlin as to how to increase the sensitivity of a methylation analysis assay.

Claims 17 and 18 are patentable over Berlin for at least the same types of reasons discussed in connection with claim 1.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 16 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Berlin (AU 200018565 B2, published June 2000)."

Without acquiescing in the propriety of the rejection, Applicants note that claim 16 has been canceled in this paper. Therefore, the rejection is moot and should be withdrawn.

Claims 7-10 and 19-20 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Berlin (AU 200018565 B2, published June 2000) in view of Herman et al (US 6,265,171)." In support of the rejection, the Patent Office states the following:

> The teachings of Berlin are discussed above. Berlin does not discuss the method where the detection is performed by nucleic acid amplification. She is also silent to the source of the DNA with respect to body fluids.
>
> However, Herman teaches a method of detecting cytosine methylation patterns by converting nonmethylated cytosines to uracil, then, amplifying and detecting the converted DNA using methylation specific primers (see abstract and col. 3, col. 4, lines 29-48; col. 5-6; Fig. 2A-E; and col. 9, lines 51-67). Herman also teaches samples obtained from serum, plasma, urine, sputum or other body fluids of an individual (see col. 7, lines 59-62).
>
> One of ordinary skill in the art would have been motivated to modify the method of Berlin to detect using methylation specific amplification, as well as use samples obtained from body fluids because it was conventional in the art at the time of the invention to use methylation specific amplification to detect cytosine methylation patterns from body fluid samples, as demonstrated by Herman et al. Since Berlin demonstrates the benefits of detecting methylated cytosines following conversion of DNA, and enzymatic cleavage in human samples, and Herman et al demonstrate that it was conventional in the art at the time of the invention to detect via methylation specific primer pairs in amplification of DNA from body fluid samples, it would have been obvious to one skilled in the art to substitute one detection method for the other to achieve the predictable result of detecting methylated cytosines in DNA following DNA conversion in body fluid samples.

Insofar as the rejection relates to claim 8, the rejection is moot in view of Applicants' cancellation of claim 8 in this paper. Insofar as the rejection relates to claims 7, 9, 10, 19 and 20, Applicants respectfully traverse the subject rejection.

Claims 7, 9, 10, 19 and 20 depend from claim 1. Claim 1 is patentable over Berlin for at least the reasons discussed above. Herman et al. does not cure all of the deficiencies of Berlin with respect to claim 1. Therefore, based at least on their respective dependencies from claim 1, claims 7, 9, 10, 19 and 20 are patentable over the combination of Berlin and Herman et al.

Moreover, Applicants wish to add the following comments regarding Herman et al.: Herman et al. addresses the question of background DNA in a broader sense. In order to be able to detect a methylation in a particular DNA sequence, Herman et al. teaches performing an amplification reaction on the basis of primers that are specific for either the methylated state of the DNA, or for the unmethylated state of the DNA. When the methylated state is to be detected, for example, the unmethylated state of the DNA can be considered as the background DNA. Since the primer used would not allow for the amplification of the unmethylated state, this background DNA would not be amplified. It is important to note that, in the Berlin approach, the background DNA is still present in an amplification reaction. Therefore, any mispairings that occur on the background DNA also allow for the generation of an amplification product, thereby introducing an error into the assay. This problem is solved by the present invention, which eliminates the background DNA during the amplification procedure, thereby making the assay more sensitive.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 11 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Berlin (AU 200018565 B2, published June 2000) in view of Gitan et al., "Methylation-Specific Oligonucleotide Microarray: A New Potential for High-Throughput Methylation Analysis," Genome Research, 2002, Vol. 12, No. 1, pp. 158-164." In support of the rejection, the Patent Office states the following:

> The teachings of Berlin are discussed above. Berlin does not discuss the method where the detection in step d) is made by means of a microarray.
>
> However, the use of microarrays to detect methylated cytosines in DNA was well known in the art at the time of the invention (see Gitan, abstract). Therefore, one of skill in the art would have been motivated to use a microarray during the detection step since Gitan demonstrates the benefits of using microarrays to detect methylation patterns in DNA. Since Berlin demonstrates the benefits of detecting methylated cytosines following conversion of DNA, and Gitan et al. demonstrate that it was conventional in the art at the time of the invention to detect methylation patterns via microarrays, it would have been obvious to one skilled in the art to substitute one detection method for the other to achieve the predictable result of detecting methylated cytosines in DNA following DNA conversion.

Applicants respectfully traverse the subject rejection. Claim 11 depends from claim 1. Claim 1 is patentable over Berlin for at least the reasons discussed above. Gitan et al. does not cure all of the deficiencies of Berlin with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 11 is patentable over the combination of Berlin and Gitan et al.

Moreover, Applicants wish to add the following comments regarding Gitan et al.: Gitan et al. also does not reduce or eliminate the background DNA in the analysis method for methylated DNA. Instead, this kind of reduction or elimination of background DNA might also not be necessary in this case as Gitan et al. teaches a method that is based on the use of a microarray (see abstract on page

158 of Gitan et al.). Microarrays are used in the art in exactly these situations, where a target sequence of DNA is present amongst very similar DNA sequences. Therefore, a person studying Gitan et al. would not have felt the need to reduce the background DNA.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 13-15 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Berlin (AU 200018565 B2, published June 2000) in view of Bazar et al, "Mutation Identification DNA analysis system (MIDAS) for detection of known mutations," Electrophoresis, 1999, Vol. 20, pp. 1141-1148 (of record)." In support of the rejection, the Patent Office states the following:

> The teachings of Berlin are discussed above. Although Berlin discusses using a DNA repair enzyme, MutS, to recognize and cleave mismatched base pairs in pretreated DNA, she does not discuss the DNA repair enzyme being Mut Y, Mug protein, DNA glycosylase, or TDG enzyme [claim 13], or a heat-stable enzyme [claim 14], or specifically, TDG enzyme [claim 15].
>
> However, the use of DNA repair enzymes to detect mismatched or erroneously base paired DNA via detection of fragments cleaved by such enzymes was well-known in the art at the time of the invention. Bazar et al. demonstrate using DNA glycosylase and thermostable TDG enzyme in such reactions (see abstract, pg. 1142, "Thermophilic MIDAS reaction," Figure 1; pg. 1144 "Detection of A/A mismatch with E. Coli Endo V").
>
> One of ordinary skill in the art would have been motivated to modify the method of Berlin to different DNA repair enzymes, such as Mut Y, Mug protein, DNA glycosylase, or themostable TDG enzyme because it was conventional in the art at the time of the invention to use such repair enzymes in reactions to recognize and cleave erroneously base paired DNA in detection of, erroneously base paired DNA, as demonstrated by Bazar et al. Since Berlin demonstrates the benefits of detecting methylated cytosines using a DNA repair enzyme, and Bazar et al demonstrate that it was conventional in the art at the time of the invention to use DNA repair enzymes, DNA glycosylase, and thermostable TDG enzyme in erroneously base paired DNA detection reactions, it would have been

> obvious to one skilled in the art to substitute one DNA repair enzyme for the other to achieve the predictable result of using DNA repair enzymes to detect methylated cytosines in DNA following chemical conversion of DNA.

Applicants respectfully traverse the subject rejection. Claims 13-15 depend ultimately from claim 1. Claim 1 is patentable over Berlin for at least the reasons discussed above. Bazar et al. does not cure all of the deficiencies of Berlin with respect to claim 1. Therefore, based at least on their respective dependencies from claim 1, claim 13-15 are patentable over the combination of Berlin and Bazar et al.

Moreover, Applicants wish to add the following comments regarding Bazar et al.: Bazar et al. teaches a strategy for DNA mutation detection that is not related to DNA methylation analysis (see abstract of Bazar et al.). According to Bazar et al., mismatching labeled probes are used to detect a point mutation in a target DNA (see figure 1 on page 1143). After the hybridization of the probe with the DNA containing the point mutation has occurred, a DNA glycosylase removes the sugar moiety on one strand at the DNA base pair mismatch. The resulting apyrimidinic or purinic site is then cleaved. The cleaved probes can then be detected, which allows one to draw a conclusion about the presence or absence of the point mutation of the DNA that was investigated. Again, the Bazar approach tries to detect a DNA with a point mutation in a population of DNA molecules without elimination or reduction of the background DNA. Therefore, Bazar et al. would not have provided a person of ordinary skill in the art with any hint as to how to arrive at the present invention.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on _____

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